PRECLINICAL PHARMACOKINETIC REPORT

Developmental Biology and Solid Tumor Program

P-PKSR Study 128786-1349772

STUDY TITLE:

SCREENING PLASMA PHARMACOKINETICS OF TAZEMETOSTAT IN FEMALE ATHYMIC NUDE MICE AFTER A SINGLE ORAL DOSE

SHORT TITLE:	**Tazemetostat Screening Plasma PK (SPPK)**
TEST ARTICLE:	Tazemetostat
SECTION:	Nonclinical Pharmacokinetics (Non-GLP)
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SJCRH SRM2 O/R:	128786-1349772 Preclinical Pharmacokinetic Shared Resource
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REPORT FORMAT:	Study Summary
REPORT STATUS:	FINAL
DATE:	2020-04-21

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Quality Statement

This non-GLP study was conducted using sound scientific principles and established techniques in accordance with the relevant guidelines and standard operating procedures (SOPs) of the Preclinical Pharmacokinetic Shared Resource (P-PKSR) and St. Jude Children's Research Hospital (SJCRH), Memphis, TN, USA. This report accurately reflects the data obtained during the course of this study.

These results represent part of an early phase preclinical pharmacology program. This study has been conducted to provide preliminary insights into the pharmacokinetic (PK) properties of the compound(s) in the indicated preclinical model(s). This study and its results are not intended to provide a comprehensive PK evaluation of the compound(s). The applied bioanalytical method was validated/qualified to support this specific study and discovery-style sample analyses.

Substantial study-to-study and inter-animal variability in preclinical PK exists. Such variability depends upon the in vivo scientists' experience, variations in compound purity and formulation, animal strains, sex and age, and other situational fixed effects (i.e. husbandry conditions, chow constituents, presence or absence of disease, concomitant drugs). As such, the actual PK, plasma or tissue compound concentrations, or equivalent dose in other studies or preclinical models may vary significantly from that reported herein.

1.0 METHODS

1.1 In Vivo Pharmacokinetic (PK) Study

The plasma pharmacokinetic (PK) profile of tazemetostat was evaluate in female athymic nude mice (Charles River), approximately 8-12 weeks in age. Tazemetostat free base (ADOOQ, Lot # L12712B002, purity 99.7%) was suspended in in 1% hydroxyethylcellulose (HEC) / 0.25% Tween 80 / 0.05% simethicone in ultrapure water, at a concentration of 20 mg/mL as a 10 mL/kg oral gavage, for a 200 mg/kg oral dose. Two survival blood samples were obtained from each mouse via retro-orbital plexus using Minivette POCT 50 µL capillary devices (Sarstedt AG, Germany), and a third final sample by cardiac puncture, all using KEDTA as the anticoagulant. To compare the results between the two sampling techniques, a paired retro-orbital blood sample was obtained from each mouse along with the terminal cardiac puncture. Samples were obtained at various times up to 24 hours post-dose, immediately processed to plasma, and stored at -80 °C until analysis. Remaining dosing suspension was submitted for verification of potency, and chemical and physical stability during the study period.

1.2 Bioanalysis

Plasma samples were analyzed for tazemetostat (ADOOQ, Lot # L12712B002, purity 99.7%) with a qualified liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay. Plasma calibrators and quality controls were spiked with solutions, corrected for salt content, prepared in acetonitrile. Plasma and tumor homogenate samples, 25 µL each, were protein precipitated with 100 µL of 100 ng/mL GSK126 (ADOOQ, Lot # B006) in acetonitrile as an internal standard. A 2 µL aliquot of the extracted supernatant was injected onto a Shimadzu LC-20ADXR high performance liquid chromatography system via a LEAP CTC PAL autosampler.

The LC separation was performed using a Waters XBridge BEH C18 XP (2.5 µm 75 x 2.1 mm) column maintained at 50 °C with gradient elution at a flow rate of 0.6 mL/min. The binary mobile phase consisted of water: 20 mM ammonium acetate; formic acid (950:50:0.25 v/v/v) in reservoir A and acetonitrile: 20 mM ammonium acetate; formic acid (950:50:0.25 v/v/v) in reservoir B. The initial mobile phase consisted of 30% B with a linear increase to 100% B in 1.25 min. The column was then rinsed for 1 min at 100% B and then equilibrated at the initial conditions for 2.0 min for a total run time of 4.25 min. Under these conditions, the analyte and IS eluted at 0.94 and 1.08 min, respectively. Analyte and IS were detected with tandem mass spectrometry using a SCIEX API 5500 Q-TRAP in the positive ESI mode with the following mass transitions were monitored: 573.30 -> 486.30 for tazemetostat and 527.30 -> 392.20 for GSK126.

The method qualification and bioanalytical runs all passed acceptance criteria for non-GLP assay performance. A linear model ($1/X^2$ weighting) fit the calibrators across the 1 to 500 ng/mL range, with a correlation coefficient (R) of 0.9985. The lower limit of quantitation (LLOQ), defined as a peak area signal- to-noise ratio of 5 or greater verses a matrix blank with IS, was 1 ng/mL. Sample dilution integrity was confirmed. For the plasma matrix, the intra-run precision and accuracy was ≤ 5.81% CV and 93.6% to 99.3%, respectively.

1.3 Pharmacokinetic (PK) Analysis

Tazemetostat plasma Ct data were grouped by nominal time point, and the mean Ct values were subjected to noncompartmental analysis (NCA) using Phoenix WinNonlin 8.1 (Certara USA, Inc., Princeton, NJ). The extravascular model was applied, was applied, and area under the Ct curve (AUC) values were estimated using the "linear up log down" method. The terminal phase was defined as at least three time points at the end of the Ct profile, and the elimination rate constant (Kel) was estimated using an unweighted log-linear regression of the terminal phase. The terminal elimination half-life (T1/2) was estimated as 0.693/Kel, and the AUC from time 0 to infinity (AUCinf) was estimated as the AUC to the last time point (AUClast) + Clast (predicted)/Kel. Other parameters estimated included observed maximum concentration (Cmax), time of Cmax (Tmax), concentration at the last observed time point (Clast), time of

Clast (Tlast), apparent clearance (CL/F = Dose/AUCinf), and apparent terminal volume of distribution (Vz/F). The paired retro-orbital and cardiac sample results from each mouse upon termination were analyzed after natural log transformation with a linear mixed effect model in R. All observations were used in summary statistics and PK analyses.

2.0 RESULTS

Tazemetostat plasma Ct data displayed moderate-to-high variability between and within mice, with CVs of 4.67% to 127%. The oral absorption of tazemetostat was rapid, with a Tmax demonstrated at 30 minutes post-dose. Plasma concentrations declined slowly in the first 8 hours after dosing, and diminished significantly thereafter through 24 hours. The apparent oral clearance of tazemetostat was high at 127 mL/min/kg, and in excess of murine hepatic blood flow. The apparent terminal volume of distribution of tazemetostat was also very high at 24.9 L/kg. The apparent terminal half-life for tazemetostat was 2.26 hr. The oral bioavailability of tazemetostat in this current study is unknown, but has previously been reported to be 55% in mice with a 10 mg/kg oral dose [1]. Given previous experience, multi-phasic, solubility or dose-limited absorption is anticipated with tazemetostat dosages above 50 mg/kg in mice. The suspension was adequate and stable over the 3-day usage period, with a measured concentration of 19.2 mg/mL and a CV of 3.5% between strata replicates.

The tazemetostat plasma PK in this study conducted by CIVIT personnel is highly similar to previous studies by the DBSTP (RPT.133410-1396203). Tazemetostat plasma Cmax and AUCinf values were 16% lower and 32% higher respectively in the current study, with Ct profiles being nearly identical. The within-mouse paired retro-orbital bleed results were not significantly different than the cardiac punctures (P = 0.7). This is in contrast to previous findings with abemaciclib (RPT.124103-1295433) and LY3020414 (RPT.130292-1364586), where the retro-orbital bleeds were approximately 14% and 5% lower, respectively. These differences are most likely to be compound-dependent, but also secondary to the technique and devices used for collections, or differences in drug distribution at the sampling sites. Notwithstanding, the biases remain within 15%, within typical bioanalytical assay error, and can be considered negligible.

A clinically relevant dose (CRD) of tazemetostat 225 mg/kg PO BID continues to be recommended in mice (RPT.133410-1396203). However, doses in mice ranging from 200 to 300 mg/kg BID could be considered clinically relevant by unbound plasma AUCs.

3.0 REFERENCES

1. Kuntz KW, Campbell JE, Keilhack H, Pollock RM, Knutson SK, Porter-Scott M, Richon VM, Sneeringer CJ, Wigle TJ, Allain CJ, Majer CR, Moyer MP, Copeland RA, Chesworth R. The Importance of Being Me: Magic Methyls, Methyltransferase Inhibitors, and the Discovery of Tazemetostat. J Med Chem. 2016 Feb 25;59(4):1556–64.

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4.0 TABLES, LISTINGS, AND FIGURES (TLFS)

Figure 4.1: Tazemetostat Ct Summary (Mean, SD, N) by Group

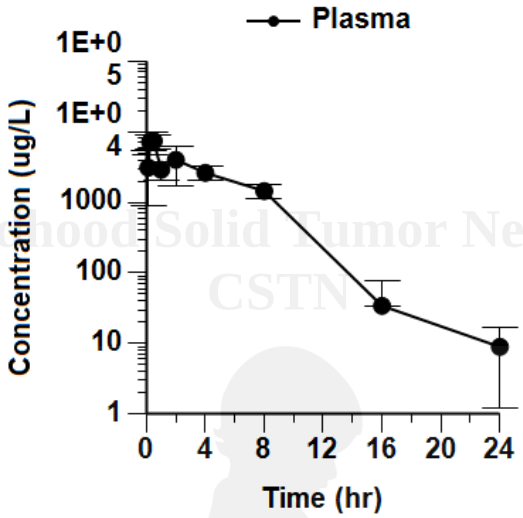

Table 4.1: NCA PK Parameter Estimates of Tazemetostat by Group

		Analyte
		Tazemetostat
		Group
		Plasma
Parameter	Units	Estimate
Cmax	ug/L	7400
Tmax	hr	0.500
AUClast	hr*ug/L	26100
AUCinf	hr*ug/L	26200
Kel	1/hr	0.307
T1/2	hr	2.26
CL/F	L/hr/kg	7.64
Vz/F	L/kg	24.9
Clast	ug/L	8.99
Tlast	hr	24.0

Table 4.2: Full Summary Statistics of Tazemetostat Ct Data by Group

		Analyte
		Tazemetostat
		Group
		Plasma
Time (hr)		Concentration (ug/L)
0.125	N	3
	Mean	3110
	SD	2240
	Min	1180
	Median	2600
	Max	5560
	CV%	71.8
	Geometric Mean	2570
	CV% Geometric Mean	91.1
0.250	N	3
	Mean	7180
	SD	2520
	Min	4900
	Median	6770
	Max	9880
	CV%	35.1
	Geometric Mean	6890
	CV% Geometric Mean	36.3
0.500	N	3
	Mean	7400
	SD	1680
	Min	6350
	Median	6510
	Max	9340
	CV%	22.7
	Geometric Mean	7280
	CV% Geometric Mean	21.8
1.000	N	3
	Mean	2900
	SD	836
	Min	1970
	Median	3160
	Max	3580
	CV%	28.8
	Geometric Mean	2810
	CV% Geometric Mean	32.4
2.000	N	3
	Mean	4010

Tazemetostat Screening Plasma PK (SPPK)

		Analyte
		Tazemetostat
		Group
		Plasma
Time (hr)		Concentration (ug/L)
	SD	2320
	Min	2410
	Median	2960
	Max	6670
	CV%	57.8
	Geometric Mean	3620
	CV% Geometric Mean	58.0
4.000	N	3
	Mean	2620
	SD	571
	Min	2100
	Median	2550
	Max	3230
	CV%	21.7
	Geometric Mean	2580
	CV% Geometric Mean	21.9
8.000	N	6
	Mean	1450
	SD	319
	Min	1010
	Median	1510
	Max	1770
	CV%	22.0
	Geometric Mean	1420
	CV% Geometric Mean	23.7
16.000	N	6
	Mean	34.2
	SD	43.6
	Min	3.14
	Median	9.15
	Max	93.0
	CV%	127
	Geometric Mean	13.8
	CV% Geometric Mean	307
24.000	N	6
	Mean	8.99
	SD	7.78
	Min	0.700
	Median	8.93
	Max	17.7

		Analyte
		Tazemetostat
		Group
		Plasma
Time (hr)		Concentration (ug/L)
	CV%	86.6
	Geometric Mean	4.67
	CV% Geometric Mean	297

Table 4.3: Tazemetostat Ct Data Listings by Subject, Analyte, Group, and Time

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
M1	Tazemetostat	Plasma	0.13	5559.70
M1	Tazemetostat	Plasma	1.00	3162.70
M1	Tazemetostat	Plasma	16.00	93.02
				87.69
M2	Tazemetostat	Plasma	0.13	2603.30
M2	Tazemetostat	Plasma	1.00	3576.70
M2	Tazemetostat	Plasma	16.00	3.14
				3.21
M3	Tazemetostat	Plasma	0.13	1175.20
M3	Tazemetostat	Plasma	1.00	1966.40
M3	Tazemetostat	Plasma	16.00	9.31
				9.00
M4	Tazemetostat	Plasma	0.25	9878.80
M4	Tazemetostat	Plasma	2.00	6669.00
M4	Tazemetostat	Plasma	24.00	16.91
				5.27
M5	Tazemetostat	Plasma	0.25	4895.10
M5	Tazemetostat	Plasma	2.00	2957.60
M5	Tazemetostat	Plasma	24.00	17.74
				12.58
M6	Tazemetostat	Plasma	0.25	6767.30
M6	Tazemetostat	Plasma	2.00	2409.10
M6	Tazemetostat	Plasma	24.00	0.74
				0.70
M7	Tazemetostat	Plasma	0.50	6510.20
M7	Tazemetostat	Plasma	4.00	3230.50
M7	Tazemetostat	Plasma	8.00	1774.70
				1477.40
M8	Tazemetostat	Plasma	0.50	9341.70
M8	Tazemetostat	Plasma	4.00	2545.60
M8	Tazemetostat	Plasma	8.00	1756.10

Subject	Analyte	Group	Time (hr)	Concentration (ug/L)
				1548.10
M9	Tazemetostat	Plasma	0.50	6353.80
M9	Tazemetostat	Plasma	4.00	2097.20
M9	Tazemetostat	Plasma	8.00	1132.40
				1005.30

Table 4.4: Tazemetostat Ct Summary (Mean, SD, N) by Group

Variable	Units	Analyte	Group	Time (hr)	Mean (ug/L)	SD (ug/L)	N
Concentration	ug/L	Tazemetostat	Plasma	0.13	3112.73	2236.20	3.00
Concentration	ug/L	Tazemetostat	Plasma	0.25	7180.40	2517.40	3.00
Concentration	ug/L	Tazemetostat	Plasma	0.50	7401.90	1681.74	3.00
Concentration	ug/L	Tazemetostat	Plasma	1.00	2901.93	836.22	3.00
Concentration	ug/L	Tazemetostat	Plasma	2.00	4011.90	2317.40	3.00
Concentration	ug/L	Tazemetostat	Plasma	4.00	2624.43	570.75	3.00
Concentration	ug/L	Tazemetostat	Plasma	8.00	1449.00	318.79	6.00
Concentration	ug/L	Tazemetostat	Plasma	16.00	34.23	43.59	6.00
Concentration	ug/L	Tazemetostat	Plasma	24.00	8.99	7.78	6.00

5.0 ATTACHED FILES

Attached File 5.1 Tazemetostat Screening Plasma PK CIVIT.docx – *Final in vivo study plan as executed*

Attached File 5.2 Terminal Tazemetostat PK.pdf – *Submitted in vivo study digital data collection form (DCF)*

Attached File 5.3 Tazemetostat Screening Plasma PK TLFs.docx – *Report TLFs as a Word document for manipulation, plotting, and further presentation*